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Changes of Benzo(a)pyrene Contents in Smoked Fish during Storage

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ABSTRACT

The paper refers to the determination of benzo(a)pyrene (BaP) contents in smoked fish by an HPLC method. Samples were analyzed after saponification and purification on Florisil and Separon SGX C-18, using selective fluorescence detection for the determination of BaP in smoked fish as well as in the model system, which was kept at the same conditions.

In the model system, the BaP degradation was found to be directly proportional to the duration of influence of environmental physicochemical factors with a clearly linear BaP decay line.

On the other hand, in the case of smoked fish the BaP contents were changed in dependence on diffusion into the internal product layers where their amount was relatively stabilized due to the absence of both light and oxygen.

Measurements have shown that BaP contents cannot be considered as a constant but that a gradual decrease is taking place as a result of various factors influencing the degradation process, which in itself can attain different velocities depending on the changing properties of the meat products as well as on environmental conditions.

INTRODUCTION

Smoking of meat and meat products is one of the most ancient technologies used for processing of meat. Notwithstanding that this method has been

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recently losing its original purpose (in the past, smoking had mainly been used for preservation of meat) smoked products retain high consumer preference due to their characteristic organoleptic properties.

Smoking is defined as the process of penetration of meat products by volatiles resulting from thermal destruction of wood. The products of burning are mainly derivatives of phenol, carbonyl compounds, and other organic compounds and consist of gases as well as liquid and solid particles in aerosol form; they consist of a large number of chemical compounds (Tóth, 1983). However, in the process of preparation of technological smoke for the above purpose, other compounds are also formed which are harmful to human health; namely, polycyclic aromatic hydrocarbons (PAHs). The quality and composition of these depend on several factors, e.g. type and composition of the wood used, access and quantity of oxygen, temperature at the smoke generation, etc. (Tóth & Blaas, 1972; Potthast, 1978). The temperature of generation is one of the key factors which influences levels of PAHs in the smoke. There is a linear dependence between increasing concentration of PAHs and increasing temperature of smoke generation. This important fact has been clearly demonstrated by Tóth and Blaas (1972).

The PAH contents can also be increased via other environmental factors (Kipper & Flemmig, 1989).

However, the differences in PAH contents are also caused by technological arrangements in the smoking units (insertion of smoke conduits between the generator and smoke house) (Potthast, 1978) and by the selected technology—the use of cold or hot smoke (Hamm & Potthast, 1976).

A characteristic PAH is benzo(a)pyrene (BaP). Although the BaP content is far from a substantial portion of the total PAHs, Andelman and Suess (1970) noted that it constitutes between 1 and 20% of the total carcinogenic PAHs and BaP is indeed one of the most potent carcinogens (Howard & Fazio, 1980). Moreover, it is well known that BaP can be found everywhere as a typical product of burning. For these reasons BaP is generally considered as the indicator of occurrence and of total assessment of health hazard in food products where the risk of PAH contamination exists.

High performance liquid chromatography (HPLC) has been found to be very suitable for the determination of PAH in a variety of smoked meat products (Gertz, 1981; Lawrence & Weber, 1984; Simko *et al.*, 1989). The same method has also been used for the quantification of BaP and other PAHs in various commodities including vegetable oils, spices, tea herbs, spice oleoresins, coffee and coffee extract (Stijve & Hischenhuber, 1987).

This work has been based upon the assumption that the content of PAHs in smoked meat products is a dynamic value, which depends on actual concrete conditions. Generally, contents of PAHs in an environment can be influenced by several physicochemical factors; for example, presence of

antioxidants, light, oxygen, temperature, etc. This paper deals with the study of BaP contents decreases in smoked fish during storage, using the HPLC method with fluorescence detection.

MATERIALS AND METHODS

The samples, i.e. smoked fish, were taken immediately after termination of the process of smoking and the BaP content was determined. Next the samples were freely hung in the laboratory under unlimited access of oxygen and daylight at 18°C. Then, the BaP content was determined after 1, 2, 3, 4, 6 and 7 days.

Sample preparation

Thirty grams of sample were saponified with a mixture of 11·2 g KOH, 90 ml methanol and 10 ml water, for 3 h under reflux. Next cooled samples were extracted three times with 50 ml of cyclohexane each, and the cyclohexane layer was separated. The cyclohexane extracts were combined and 100 ml of 10% by weight Na_2WO_4 water solution was added for precipitation of lipoproteins. After separating the cyclohexane layer, the precipitate was decanted using 100 ml of cyclohexane which then, after separation, was also added to the main portion. Further precipitations of the cyclohexane extract, with twice 100 ml Na_2WO_4 solution, were used to remove all residual lipoproteinic matter. Then the cyclohexane extract was dried over anhydrous Na_2SO_4 and evaporated to a volume of 1 ml using a rotary vacuum evaporator. The concentrated cyclohexane extract was applied to the top of a 2·5 g Florisil column (60–100 mesh) and eluted with 150 ml of cyclohexane. The eluate was collected and rotary-evaporated to near dryness. The residue from the above was dissolved in methanol and made up to 1 ml volume. A silica cart (firm Tessek, Czechoslovakia) filled with Separon SGX C 18 silica gel (40 μm) was washed with 10 ml of methanol, and dried with 3 ml of air. Following this, the methanolic solution of the PAH fraction was applied to the silica cart and eluted with 3 ml of methanol. The eluate was subsequently evaporated to 1 ml, and injected into a liquid chromatograph.

HPLC conditions

HPLC was performed isocratically on a Separon SGX C 18 reverse phase column (5 μm, length 30 cm and i.d. 3 mm, firm Tessek, Czechoslovakia) at ambient temperature. Mobile phase was a mixture of acetonitrile and water

3:1 (v/v) with a flow rate of 1·15 ml/min. Instrumentation consisted of a high pressure pump HPP 4001 and a loop injector. The effluent from the column was directed to a Perkin-Elmer fluorescence detector, which operated at 310 nm excitation wavelength and 410 nm emission wavelength. The elution time of BaP was determined by adding BaP standard solution (firm Supelco, Switzerland, No. 4-8564).

RESULTS AND DISCUSSION

First, the efficiency of the extraction and cleanup method must be tested by recovery studies. Samples of smoked fish were spiked with amounts of BaP which corresponded to the level equivalent of 1·0 μg BaP per kilogram of sample. The results are shown in Table 1.

The detection limit of this method at the given conditions is 0·03 μg/kg.

Measured values calculated for BaP contents in 1 kg of the sample are shown in Table 2 and graphically represented in Fig. 1. The results show that the dependence as represented by Fig. 1 is not linear as might have been assumed. A linear process of BaP degradation was only found in the model system, which was carried out simultaneously with the experimental work as follows: standard BaP solution (2 mg BaP in 1 litre of methanol) was transferred by micro syringe (firm Hamilton, Switzerland) into far UV silica cells and, after evaporation of the solvent, BaP was exposed to the same conditions as used for the smoked fish samples. The contents of the cells were analyzed for presence and amount of BaP at the same time intervals. Table 3

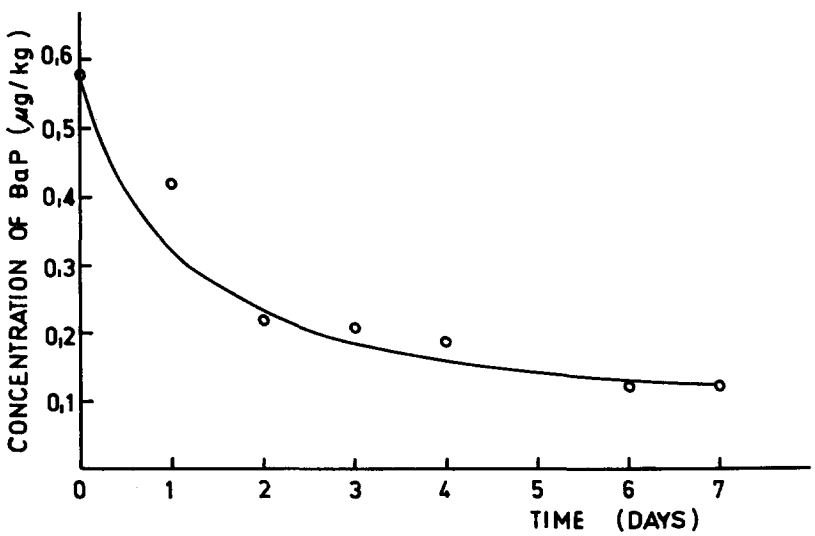

Fig. 1. The dependence of BaP concentration upon time in smoked fish.

TABLE 1
Per cent Recoveries of BaP from Spiked Smoked Fish

Sample	*Average recovery*[a] (%)	*Standard deviation* (%)
Fish I	77·6	8·2
Fish II	79·8	6·7
Fish III	74·8	7·9

[a] Average of triplicates.

TABLE 2
Changes of BaP Concentration in Smoked Fish during Storage

Time of storage (*days*)	*BaP concentration*[a] (*μg/kg*)
0	0·58
1	0·42
2	0·22
3	0·21
4	0·19
6	0·12
7	0·12

[a] Averages of duplicates.

TABLE 3
Changes of BaP Amount in Model System during Storage

Time of storage (*days*)	*BaP amount* (*μg*)
0	1·00
2	0·78
3	0·62
4	0·45
5	0·31
7	0·05

shows the results of measurements of BaP, corresponding to the respective time intervals whereas Fig. 2 shows the dependence of BaP decrease with time. These results show that, in this case, the BaP degradation depth is directly proportional to the duration of influence of environmental physico-chemical factors. The results also show that the BaP degradation is a typical photolytic decomposition. In smoked fish, the BaP degradation process is

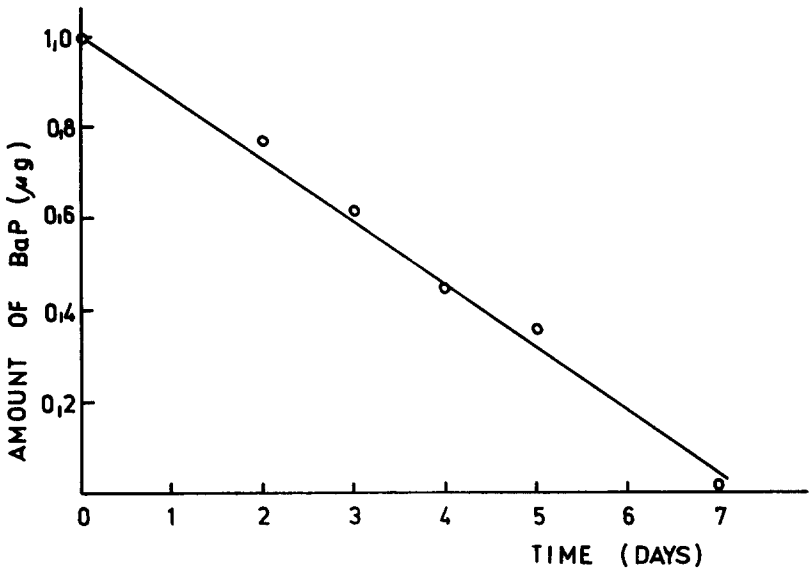

Fig. 2. The dependence of BaP amount upon time in the model system.

far more complicated. The data indicate that additional factors, defined by the characteristic properties of the smoked products themselves, affect the degradation velocity and depth. Inmediately upon termination of the process of smoking, BaP was only found on the product surface (Table 4). With increasing time, however, not only degradation but also diffusion into internal layers of the smoked fish were taking place, with subsequent stabilization of the final amount inside the product due to the absence of light and oxygen, as shown in Fig. 1. In all, the BaP concentration has, in this case, decreased from the initial 0·58 μg/kg to the final 0·12 μg/kg. This is interesting from the particular viewpoint of a country where maximum BaP

TABLE 4
Concentration of BaP in the Surface Layer (Hide) and in the Internal Layer (Meat)

Time lapse	*BaP concentration*[a] (μg/kg)
Immediately after smoking	
Surface layer	10·61
Internal layer	0·00
After 7 days' storage	
Surface layer	1·28
Internal layer	0·09

[a] Averages of duplicates.

quantities in smoked meat products have been limited by a health service legislation. A paradoxical situation might arise that a product containing, for example, 1·2 μg/kg of BaP immediately after the smoking process, and consequently failing to meet hygienic standards of the FRG, can, after a certain duration of storage, well meet these specifications as a result of partial BaP degradation. Another question arises in connection with the diffusion velocity of BaP into the meat products. Generally, diffusion is proceeding in accordance with the Fick laws where the second Fick law describes changes in the concentration of given compounds in time dependence by the equation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

The diffusion coefficient, D, depends not only upon the compound undergoing diffusion and the temperature, but upon the environmental conditions as well, and—to a lesser extent—upon pressure and concentration (Kellö & Tkáč, 1977). This fact virtually eliminates the possibility of determining the diffusion coefficient for smoked meat products, the composition of which is extremely complex. In addition to this, BaP present in the external layers, or on the surface, respectively, is unstable in character which further complicates the issue. In this connection it is interesting to note that the use of packaging materials with defined microopening sizes could be of importance with respect to their permeability for gases, water, and sensory active compounds present in wood smoke (phenolic derivatives, carbonyls, organic acids). Taking the substantially larger size of PAH molecules into account, these could be virtually prevented from diffusion into the deeper product layers, thus contributing significantly to the elimination of these compounds in smoked meat products and to their quality.

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